

centrifugal filtration (Zhu et al., 1985). SfiI adaptors (5' GTTGGCCTTTT, SEQ ID NO:115) were attached to the DNA ends (blunt) to facilitate cloning of the fragments into the vector. After ligation of these adaptors to the DNA fragments a second size-fractionation was performed on an agarose gel. The small genomic DNA fragments were cloned upstream of the GAL1 promoter in the vector pGAL1PSiST-1. Qiagen-purified pGAL1PSiST-1 plasmid DNA was digested with SfiI and the largest vector fragment eluted from the gel by centrifugal filtration (Zhu et al., 1985). The ligation mix was electroporated to MC1061 (...) E. coli cells. -

Kindly amend the paragraph beginning on line 10, page 24 to read:

--Inverse PCR was performed on 1 µl of the precipitated ligation reaction using library vector specific primers (Figure 1) (3pGALSistPCR: 5' GAG-GGC-GTG-AAT-GTA-AGC-GTG 3' (SEQ ID NO:16) and 5pGALNistPCR: 5'GAG-TTA-TAC-CCT-GCA-GCT-CGA-C 3' (SEQ ID NO:17) for the genomic library; 3pGALNistPCR: 5' TGA-GCA-GCT-CGC-CGT-CGC-GC 3' (SEQ ID NO:18) and 5pGALNistPCR for the cDNA library; all primers from Eurogentec) for 30 cycles each consisting of (a) 1 min at 95 °C, (b) 1 min at 61 (or 57 °C for the cDNA library primers), and (c) 3 min at 72 °C. In the reaction mixture 2.5 units of Taq polymerase (Boehringer) with TaqStart antibody (Clontech) (1:1) were used, and the final concentrations were 0.2 µM of each primer, 3 mM MgCl₂ (Perkin Elmer Cetus) and 200 µM dNTPs (Perkin Elmer Cetus). All PCR reactions were performed in a Robocycler (Stratagene).--

Kindly amend the paragraph beginning on line 26, page 24 to read:

--PCR analysis is also performed on genomic DNA isolated from the transformants using primers 3pGALSistPCR and 5pGALNistPCR for the genomic library transformants and using primers

oligo23': 5' TGC-AGC-TCG-ACC-TCG-AGG 3' (SEQ ID NO:119) and
oligo25: 5' GCG-TGA-ATG-TAA-GCG-TGA-C 3' (SEQ ID NO:20) ($T_{\text{hybr}} =$
53 °C) for the cDNA library transformants. --

**Kindly amend the paragraph continuing on the top of page 28
through line 28 of page 28 to read:**

--(Perkin Elmer Cetus). Cells were grown to $OD_{600} \sim 1.0$ and
total RNA was prepared using the RNeasy midi kit (Qiagen)
according to the manufacturer's instructions. All RNA samples
were DNaseI (Boehringer-Mannheim, RNase-free)-treated at 20 U/ μ g
in 50 μ l solution for 40 min at ambient temperature,
phenol/chloroform-extracted and precipitated. Pellets were
dissolved in 20 ml MilliQ water (Millipore) and RNA
concentrations were determined spectrophotometrically. First-
strand cDNA synthesis was performed in a final volume of 20 μ l
containing 1x Superscript RT buffer (Life Technologies), 10 mM
DTT, 125 μ M of each dNTP, 50 μ M hexamer primers (Life
Technologies) and 1 mg RNA. Mixtures were incubated for 10 min.
at ambient temperature and 1 μ l was removed and diluted 1:4 for
the non-amplification control (NAC); 20 U Superscript reverse
transcriptase (Life Technologies) was added and the reaction was
incubated for 1 hour at 42 °C. The enzyme was inactivated for 10
min at 70°C. PCR reactions were set up in triplicate for all
genes and contained 5 μ l PCR buffer A, 4 mM $MgCl_2$, 200 μ M each of
dATP, dGTP, dCTP and 400 μ M dUTP, 250 nM fluorogenic probe (for
RNR1: 5' TGA-TCT-CAA-AAA-GTG-CTG-GAG-GAA-TCG-GT 3', SEQ ID
NO:121), 0.5 U UNG, 1.25 U AmpliTaq Gold, 16.75 μ l H_2O , 300 nM of
appropriate FORWARD (for RNR1: 5' CGA-CAC-TTT-GAA-ATC-GTG-TGC-T
3', SEQ ID NO:122) and REVERSE (for RNR1: 5' GCA-CCG-GTA-GAA-
CGA-ATG-TTG 3', SEQ ID NO:123) PCR primers, 1 μ l of the RT
reaction mixture. --